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## Analysis of Competition in Soil among 2,4-Dichlorophenoxyacetic Acid-Degrading Bacteria

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**Competition among indigenous and inoculated 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria was studied in a native Kansas prairie soil following 2,4-D additions. The soil was inoculated with four different 2,4-D-degrading strains at densities of  $10^3$  cells per g of soil; the organisms used were *Pseudomonas cepacia* DBO1(pJP4) and three Michigan soil isolates, strain 745, *Sphingomonas paucimobilis* 1443, and *Pseudomonas pickettii* 712. Following 2,4-D additions, total soil DNA was extracted and analyzed on Southern blots by using a *tfdA* gene probe which detected three of the strains and another probe that detected the fourth strain, *S. paucimobilis* 1443, which belongs to a different class of 2,4-D degraders. *P. cepacia* DBO1(pJP4), a constructed strain, outcompeted the other added strains and the indigenous 2,4-D-degrading populations. The *S. paucimobilis* population was the secondary dominant population, and strain 745 and *P. pickettii* were not detected. Relative fitness coefficients determined in axenic broth cultures predicted the outcome of competition in soil for some but not all strains. Lag time was shown to be a principal determinant of competitiveness among the strains, but the lag times were significantly reduced in mixed broth cultures, which changed the competitive outcome. Plasmids containing the genes for the 2,4-D pathway were important determinants of competitiveness since plasmid pKA4 in *P. cepacia* DBO1 resulted in the slower growth characteristic of its original host, *P. pickettii*, rather than the rapid growth observed when this strain harbors pJP4.**

One of the major goals in microbial ecology is to understand how interactions between microbial species influence their survival and abundance in nature. Of the various types of interactions between microbial populations, competition for carbon is often the major determinant of the relative levels of abundance of indigenous organisms in soil environments (3, 19). Pesticides and other xenobiotic compounds are new carbon compounds that have been introduced into the environment during the past several decades. Some of these compounds are good carbon sources for microorganisms capable of degrading them. For example, 2,4-dichlorophenoxyacetic acid (2,4-D) is known to be a growth substrate for a number of different soil microorganisms (4, 15-17). Since such synthetic compounds are usually not present in nature and their supply is under human control, they make good models to study microbial resource competition in soil.

Batch liquid culture and chemostat experiments have provided the basic background information for understanding the principles of microbial competition for common carbon sources (11, 12), but it is not clear that we can easily extrapolate from this information to predict the outcome of competition in a poorly mixed soil habitat. Furthermore, competition studies have been difficult to perform in soil because of limited techniques for enumerating different competing organisms. DNA probe methods offer the potential advantage of being able to distinguish and identify a number of competing organisms in inoculated as well as native communities without a requirement for prior selective culturing.

In this study, gene probes were used to study competition for

the growth substrate 2,4-D among inoculated and indigenous 2,4-D-degrading bacteria in a native prairie soil which had no history of cultivation or agricultural chemical use. In addition, batch liquid cultures were used to study the growth patterns of 2,4-D-degrading bacteria in axenic and mixed cultures, and the results were compared with the results obtained from the soil competition experiments. Finally, we evaluated the effects of different 2,4-D-degradative plasmids on both the growth patterns and the competitive advantage provided to the host bacteria.

### MATERIALS AND METHODS

**Bacteria and soil.** The bacterial strains used or isolated in this work and their sequence homologies to *tfd* genes from plasmid pJP4 are shown in Table 1. *Pseudomonas cepacia* DBO1(pKA4) was obtained through filter mating between *P. cepacia* DBO1 and *Pseudomonas pickettii* 712(pKA4). All *tfdA*-hybridizing strains could be distinguished by unique restriction fragments; the sizes of *EcoRI* fragments in *P. pickettii*(pKA4), strain 745, and *P. cepacia*(pJP4) were determined to be 3.5, 13.8, and 8.3 kb, respectively, in preliminary experiments.

The soil used in this study was obtained from the Konza Prairie near Manhattan, Kans. This is the largest native prairie area remaining in the United States, a 3,400-ha tract, and has never been cultivated or treated with agricultural chemicals. The results of most-probable-number (MPN) experiments indicated that there was a low background level of native 2,4-D degraders in this soil (ca. 30 cells per g). This soil was also selected because we believed that it should provide a microbial environment unlike the environment from which the inoculated strains were isolated, and therefore competition between indigenous strains and invaders (which were perhaps less fit) could be evaluated. The inoculated strains were obtained from a Michigan soil (9) which was formed from glacial till in a

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TABLE 1. Bacterial strains and plasmids

Strain	Plasmid <sup>a</sup>	<i>tfd</i> genes which hybridize to plasmid	Original habitat of isolate
<i>P. cepacia</i> DBO1(pJP4)	pJP4	<i>tfdA</i> , <i>tfdB</i> , <i>tfdC</i> , <i>tfdD</i>	Laboratory <sup>b</sup>
<i>P. cepacia</i> DBO1(pKA4)	pKA4	<i>tfdA</i>	Laboratory
745 <sup>c</sup>	pBS5	<i>tfdA</i> , <i>tfdB</i> , <i>tfdC</i> , <i>tfdD</i>	Kellogg Biological Station soil, Michigan
K17	pKO51	<i>tfdA</i> , <i>tfdB</i> , <i>tfdC</i> , <i>tfdD</i>	Konza Prairie soil, Kansas
<i>S. paucimobilis</i> 1443 <sup>c</sup>	pBS3	None	Kellogg Biological Station soil, Michigan
<i>P. pickettii</i> 712(pKA4) <sup>c</sup>	pKA4	<i>tfdA</i>	Kellogg Biological Station soil, Michigan

<sup>a</sup> 2,4-D-degradative plasmids were isolated from *P. pickettii* 712(pKA4), strain 745(pBS5), and strain K17 (pKO51). *S. paucimobilis* 1443 contains a large plasmid (pBS3), from which the *Spa* fragment was derived.

<sup>b</sup> Provided by R. Olsen, University of Michigan. The plasmid was isolated in Australia from an *Alcaligenes* strain (1).

<sup>c</sup> Isolated from the Long-Term Ecological Research Program Gene Flow plot at the Kellogg Biological Station, Hickory Corners, Mich. Isolation and characterization of the strains are described in an accompanying paper (9).

humid region, was originally forested, and is now under cultivated agricultural crops, while the Konza Prairie soil used was derived from layered limestone and shale in a semiarid region and is under native grasses. Both soils obtained were from Long-Term Ecological Research Program sites.

**Media and culture conditions.** All strains were maintained on MMO mineral medium (18) containing 2,4-D at a concentration of 500 ppm (500 µg/ml). Strains that were to be inoculated into soils and flasks were cultured to the late log phase at 30°C in Luria broth and then reinoculated into fresh Luria broth (1:50) to obtain fresh overnight cultures. The numbers of total viable cells were determined by plating appropriate dilutions of soil suspensions onto peptone-tryptone-yeast extract-glucose (PTYG) agar (9). The strains used for plasmid isolation were cultured in MMO mineral medium containing 500 ppm of 2,4-D. Plasmids were isolated by the method of Hirsch et al. (6).

**Inoculation and sampling of bacteria in soil.** Overnight cultures of four different 2,4-D-degrading bacteria, *P. cepacia* DBO1(pJP4), strain 745, *S. paucimobilis* 1443, and *P. pickettii* 712, were grown at 30°C, harvested by centrifugation at 10,000 × g for 10 min at 4°C, washed twice with an equal volume of 15 mM sodium phosphate buffer (pH 7.0), and again collected by centrifugation. The cells were resuspended in 0.1 volume of sodium phosphate buffer, kept on ice, and enumerated by using a counting chamber.

Konza Prairie soil was sifted through a 2-mm-pore-size sieve, adjusted to a water content of ca. 35% (wt/wt), and inoculated with each of the four different species at a density of  $1.2 \times 10^3 \pm 0.1 \times 10^3$  cells per g of soil. The soil was thoroughly mixed, and 400 g was transferred to each of three replicate polyethylene wide-mouth bottles (microcosms I through III). Three other replicates were not inoculated with 2,4-D-degrading bacteria (microcosms IV through VI). Inoculated and uninoculated soils were treated with 2,4-D dissolved in 0.1 M NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.0) to a concentration of 250 ppm and thoroughly mixed. The disappearance of 2,4-D from soil was monitored by high-performance liquid chromatography (10), and the soils were respiked with 2,4-D (250 ppm) after it was removed until a total of 10 cycles of degradation had been completed. At time zero (before the preparations were split into three replicates) and at the end of the second, fifth, and tenth 2,4-D treatments, a 10-g subsample from each microcosm was used for both a MPN determination (8) and a determination of the total viable counts on PTYG plates, and a 50-g subsample was used to isolate total bacterial DNA by the cell extraction method (7).

**Batch culture experiments.** Bacterial strains which were cultured, harvested, and prepared in sodium phosphate buffer

as described above were used for axenic and mixed-culture experiments. Liquid culture studies were conducted in 250-ml Erlenmeyer flasks containing 100 ml of MMO minimal medium supplemented with 500 ppm of 2,4-D. Axenic culture studies were performed in duplicate, and mixed-culture studies were performed in triplicate. Duplicate cultures were sufficient for the axenic culture studies because the maximum variation between duplicate cultures was less than 10%. The mixed-culture studies were performed with three strains, each inoculated at the same density ( $1.0 \times 10^6 \pm 0.2 \times 10^6$  cells per ml), and individual strains were distinguished and counted on the basis of distinctive colony morphologies.

To study the effects of induction of a 2,4-D pathway and the effects of culture interactions, strain 712 was first grown in Luria broth or in MMO minimal medium supplemented with 500 ppm of succinate. The culture was then harvested, washed in phosphate buffer, and shifted to 2,4-D growth medium. All cultures were incubated at 30°C and were aerated by shaking at 200 rpm with a New Brunswick model G24 environmental incubator shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Growth was measured by determining the optical density at 550 nm.

**Probe preparation and hybridization.** The *tfdA* gene probe and the *S. paucimobilis*-specific probe (*Spa* probe) were used to detect and distinguish the inoculated and indigenous 2,4-D-degrading bacteria by Southern blot analysis. The *tfdA* and *Spa* probes were prepared as described in an accompanying paper (10). The probes were used at concentrations of approximately 10<sup>6</sup> cpm per ml of hybridization fluid. Total soil bacterial DNA was digested and subjected to Southern transfer as described in an accompanying paper (10). Prehybridization, hybridization, and posthybridization washes were performed as described in another accompanying paper (9). Hybridization filters were exposed to X-Omat X-ray film (Kodak) at -70°C with an intensifying screen. The exposure times used were 1 to 4 days, depending on the intensity of the radioactive signal.

## RESULTS

**Degradation of 2,4-D in soil.** The patterns of degradation of 2,4-D in Konza Prairie soil with and without added 2,4-D-degrading bacteria are shown in Fig. 1. 2,4-D was quickly degraded without a lag period in inoculated soil maintained at a soil water content of ca. 35% (wt/wt) and incubated at room temperature (Fig. 1A). Under these conditions, it took 1 week or less for each addition of 250 ppm of 2,4-D to be degraded throughout the 10 treatments used. In the uninoculated soil samples, exposure of the indigenous 2,4-D-degrading populations in Konza Prairie soil to 2,4-D for the first time resulted in

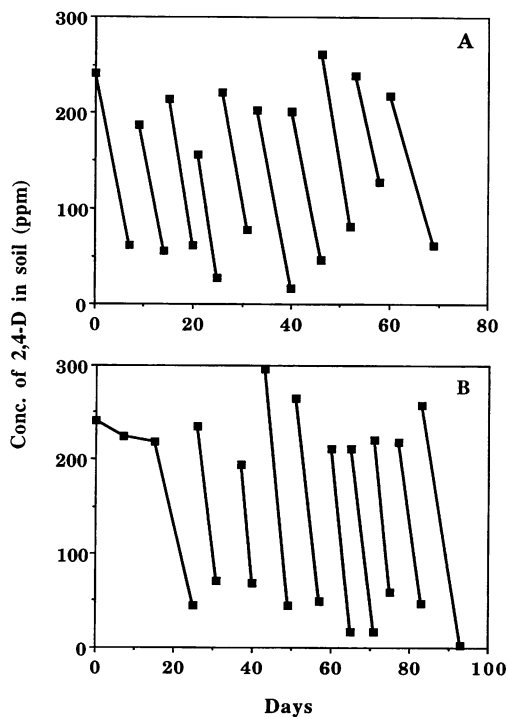


FIG. 1. Degradation of 2,4-D in soil microcosms following 10 2,4-D additions. Microcosms were inoculated with four 2,4-D-degrading strains (A) or contained only indigenous 2,4-D-degrading bacteria (B).

a lag period of about 2 weeks prior to complete degradation within 3 weeks (Fig. 1B). This lag period was not observed after the second and subsequent additions, and the 2,4-D degradation rate was similar to that observed for inoculated soil.

In soil inoculated with 2,4-D-degrading bacteria, the initial population of 2,4-D degraders contained  $7.5 \times 10^3$  cells per g of soil as measured by MPN counting (Fig. 2). By the second treatment with 2,4-D, the population density had markedly increased to  $1.8 \times 10^8$  cells per g of soil, and this population density was stably maintained throughout subsequent 2,4-D treatments. In the uninoculated soil, the initial population density detected by the MPN method was  $3.1 \times 10^1$  cells per g of soil; the value increased to  $3.1 \times 10^7$  cells per g of soil by the second addition of 2,4-D and then was maintained at this level through subsequent additions of 2,4-D (Fig. 2). Throughout the 10 treatments with 2,4-D, the population density of 2,4-D degraders detected by the MPN method was higher by a factor of 6 in inoculated soil than in uninoculated soil.

**Relationship of 2,4-D-degrading populations to total viable counts.** Addition of 2,4-D as a specific carbon source had a marked effect on the community structure (Fig. 2). In Konza Prairie soil, the initial total viable counts were  $7.8 \times 10^7$  and  $4.8 \times 10^7$  cells per g of soil in inoculated soil and uninoculated soil, respectively, and the total viable counts increased three- to fivefold after repeated 2,4-D treatments in both soil types. By contrast, the 2,4-D-degrading populations increased  $2.4 \times 10^4$ -fold and  $10^6$ -fold after repeated 2,4-D treatments in inoculated soil and uninoculated soil, respectively. Under these selective conditions, the rapidly growing populations of 2,4-D-degrading bacteria became dominant members of the total bacterial community, as indicated by the decreasing ratios of total viable counts to counts of 2,4-D-degrading bacteria and the absolute increases in viable counts (Fig. 2).

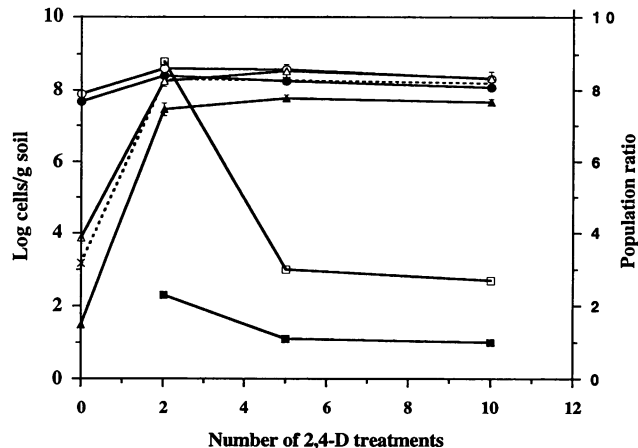


FIG. 2. Changes in the numbers of soil microorganisms in response to repeated additions of 2,4-D. Symbols:  $\Delta$  and  $\blacktriangle$ , MPN counts of 2,4-D degraders in inoculated soil and in uninoculated soil, respectively;  $\times$ , plate counts of *P. cepacia* DBO1(pJP4) on PTYG in inoculated soil;  $\circ$  and  $\bullet$ , total viable counts on PTYG in inoculated soil and in uninoculated soil, respectively;  $\blacksquare$  and  $\square$ , ratios of total viable counts to 2,4-D degrader counts in inoculated soil and in uninoculated soil, respectively. The data are the means and standard deviations for three replicate microcosms.

In inoculated soil, typical *P. cepacia* DBO1(pJP4) colonies (irregular, white, large colonies) were always detected in the greatest numbers on PTYG plates (Fig. 2), suggesting that this strain was predominant throughout the experiment. The ratio of 2,4-D-degrading bacteria to *P. cepacia* DBO1(pJP4) decreased from 5 to about 1 during the 10 2,4-D treatments (data not shown), indicating that this strain dominated the 2,4-D-degrading community as well as the total community.

**Probing total soil bacterial DNA.** Total bacterial DNAs isolated from the three replicate microcosm soils at different times were hybridized to  $^{32}\text{P}$ -labelled *tfdA* gene probe after restriction digestion, size fractionation of DNA fragments, and Southern transfer. In microcosm soils inoculated with all four 2,4-D-degrading bacteria, a single DNA hybridization band was detected in the DNA isolated after the second, fifth, and tenth treatments with 2,4-D, and no band was observed in the DNA sample from time zero (Fig. 3A). The size of the hybridized band obtained from total soil bacterial DNA digested with *EcoRI* was about 8.3 kb, which corresponded to the size of a fragment containing the *tfdA* gene in plasmid pJP4 digested with the same enzyme.

To confirm the origin of the band from soil, plasmid pJP4 DNA and total soil bacterial DNA obtained after the second 2,4-D treatment were digested with three different restriction enzymes, and the sizes of the bands that hybridized to the *tfdA* probe were compared (Fig. 4, lanes 7 through 12). Matching band patterns were obtained for the two DNA samples, indicating that the bands corresponded to *P. cepacia* DBO1(pJP4) and that this strain predominated in the soil used in this experiment. This hybridization result is consistent with the result obtained in the plate count study which indicated that *P. cepacia* DBO1(pJP4) was the predominant colony type among the added and indigenous 2,4-D degraders throughout the experiment.

When total soil bacterial DNAs from the inoculated microcosms were digested with *HindIII*, subjected to Southern transfer, and hybridized to the *Spa* probe, two bands of hybridization (5.0 and 11.3 kb) were observed with DNA

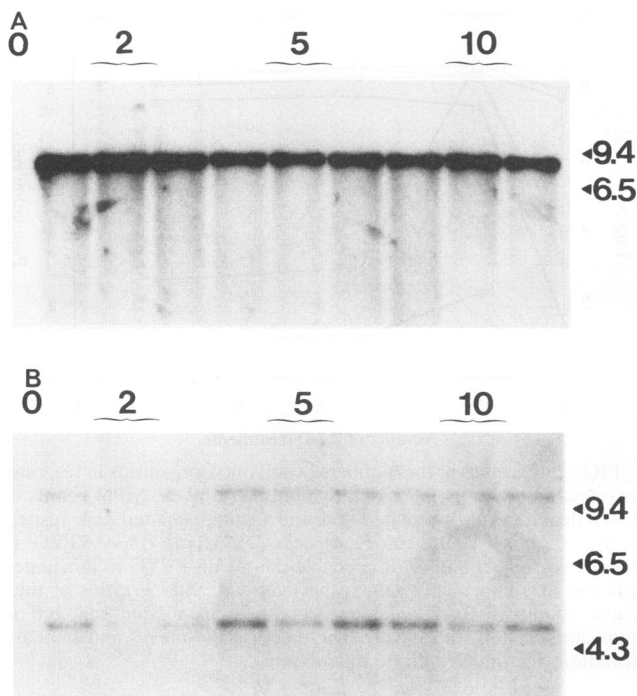


FIG. 3. Hybridization of labelled *tfdA* gene probe (A) and *Spa* probe (B) with total soil bacterial DNA. The results for three replicate microcosms containing inoculated soils are shown. Lanes 0, total soil bacterial DNA from time zero; lanes 2, total soil bacterial DNA after the second 2,4-D treatment; lanes 5, total soil bacterial DNA after the fifth 2,4-D treatment; lanes 10, total soil bacterial DNA after the tenth 2,4-D treatment. Each lane contained 1.5  $\mu\text{g}$  of total soil bacterial DNA digested with *EcoRI* (A) or *HindIII* (B).

samples isolated after the second, fifth, and tenth treatments with 2,4-D (Fig. 3B). No hybridization was observed with the DNA sample obtained at time zero. The same pattern of hybridization bands was observed on Southern blots when *S. paucimobilis* 1443 DNA was digested with the same enzyme and hybridized to the same probe (10), suggesting that this strain produced the hybridization bands in the microcosm DNA. Since each lane contained the same amount of total DNA (1.5  $\mu\text{g}$ ) and the probes were labelled to similar specific activities (ca.  $1.0 \times 10^8$  cpm/ $\mu\text{g}$ ), the weaker intensities of the latter bands indicate that *S. paucimobilis* 1443 was maintained at a lower density than *P. cepacia* DBO1(pJP4). This interpretation is further supported by the fact that *S. paucimobilis* 1443 colonies were not obvious on the PTYG plates.

In uninoculated soils, bands hybridizing to the *tfdA* probe began to appear only after five 2,4-D treatments (Fig. 5). This population was perhaps becoming prominent at this time since the bands from two of the three microcosms were weak. The intensities of the bands from the microcosm that exhibited weak hybridization increased and became similar to the intensity of the strong band from uninoculated microcosm I by the tenth 2,4-D treatment. Total soil bacterial DNA obtained after the tenth 2,4-D treatment and plasmid DNA from a new 2,4-D-degrading bacterium isolated from the same soil (designated strain K17) were digested with three different restriction enzymes and compared (Fig. 4, lanes 1 through 6). Matching band patterns were observed for these two DNA samples, suggesting that strain K17 had become dominant in the uninoculated microcosms. No band was detected when the

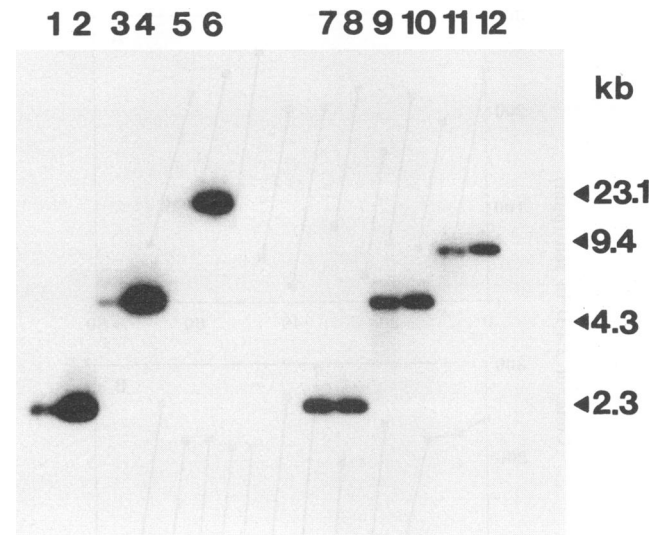


FIG. 4. Comparison of *tfdA* hybridization bands produced by total soil bacterial DNA and plasmid DNA digested with three different restriction enzymes. Lanes 7, 9, and 11, total soil bacterial DNA after the second treatment of inoculated microcosm I; lanes 8, 10, and 12, plasmid pJP4 DNA from *P. cepacia* DBO1(pJP4) after the second treatment of inoculated microcosm I; lanes 1, 3, and 5, total soil bacterial DNA after the tenth treatment of uninoculated microcosm I; lanes 2, 4, and 6, plasmid DNA from strain K17 after the tenth treatment of uninoculated microcosm I. The DNA was digested with *BglII* (lanes 1, 2, 7, and 8), *PstI* (lanes 3, 4, 9, and 10), or *EcoRI* (lanes 5, 6, 11, and 12).

uninoculated soil bacterial DNA was hybridized to the *Spa* probe.

**Growth of 2,4-D-degrading bacteria in axenic broth cultures.** To understand axenic growth patterns of 2,4-D degraders, each strain was inoculated into 2,4-D minimal medium under uninduced conditions, and the lag period, specific growth rate (2), and relative fitness coefficients (13) were determined (Table 2). Strain 745, *P. cepacia* DBO1(pJP4), and strain K17 exhibited short lag periods (<15 h) and began to grow exponentially after about 20 h of incubation (Fig. 6). By contrast, *S. paucimobilis* 1443 exhibited a longer lag period (ca. 35 h), and *P. pickettii* 712 exhibited the longest lag period (>60 h). The specific growth rates of most of these bacteria during exponential growth were similar; the exception was *P. pickettii*, whose specific growth rate was markedly lower. Strain 745 had the largest number of doublings during the first 30 h of incubation and had the highest fitness coefficient among these 2,4-D degraders, suggesting that it might be the best competitor.

The role of the host cell background versus the role of the plasmid encoding the 2,4-D-degradative genes in determining growth characteristics was evaluated by comparing the growth parameters of the same host containing two different plasmids and the growth parameters obtained with the same plasmid in two different hosts. *P. cepacia* DBO1 carrying plasmid pKA4 instead of plasmid pJP4 exhibited the lower growth rate of the original plasmid-containing host (Table 2) and a lag time intermediate between the lag times of the original host, *P. pickettii*, and the new host, *P. cepacia* (Fig. 6).

**Competition in broth cultures.** The behavior of each 2,4-D-degrading isolate in three-member broth cultures was monitored by plate counting. When Luria broth-grown strain 745, *P. cepacia* DBO1(pJP4), and K17 were inoculated together into

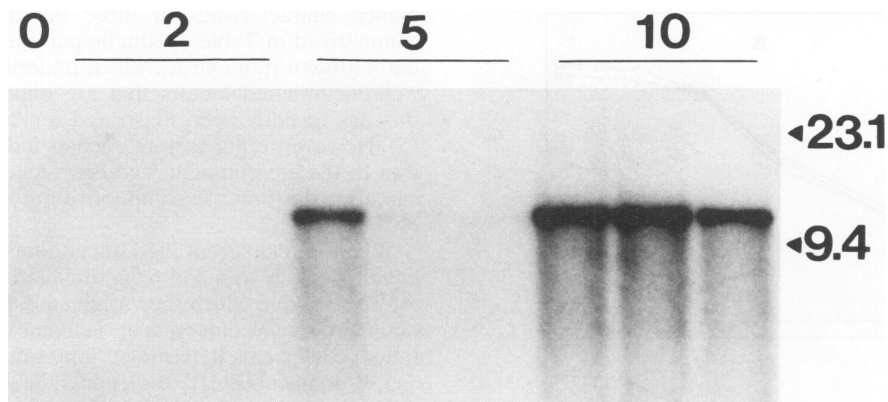


FIG. 5. Hybridization of labelled *ffdA* probe with total soil bacterial DNA from three replicate uninoculated soil microcosms. Lanes 0, total soil bacterial DNA from time zero; lanes 2, total soil bacterial DNA after the second 2,4-D treatment; lanes 5, total soil bacterial DNA after the fifth 2,4-D treatment; lanes 10, total soil bacterial DNA after the tenth 2,4-D treatment. Each lane contained 1.5  $\mu$ g of total soil bacterial DNA digested with *Hind*III.

2,4-D minimal medium at a ratio of 1:1:1, these 2,4-D degraders exhibited similar growth patterns (Fig. 7A). This result is consistent with the result obtained in the axenic growth experiment, in which all of these strains exhibited a short lag period and rapid growth. In mixed cultures containing strain 745, *S. paucimobilis* 1443, and *P. pickettii* 712 at a ratio of 1:1:1 (Fig. 7B), strain 745 multiplied quickly and slightly outgrew the other two strains in the initial phase, but after 33 h of incubation its growth rate began to decline even though 65% of the substrate remained. By contrast, *S. paucimobilis* 1443 initiated growth earlier in mixed cultures than in axenic cultures, thereby overcoming strain 745 in the last phase. This result was unexpected because, on the basis of the results of the axenic growth experiment, strain 745 should have reached stationary phase and depleted the substrate before *S. paucimobilis* 1443 and *P. pickettii* 712 began to grow. *P. pickettii* 712 also exhibited significant growth during the first 40 h of incubation in mixed cultures, whereas it did not exhibit any growth during 80 h of incubation in axenic cultures.

To further analyze the stimulatory effect of coculturing on the strains that grew slowly in axenic cultures, we grew the slowest growing strain, *P. pickettii* 712, under several cultural conditions (Fig. 8). Since the experiments described above included a nutritional shiftdown from Luria broth to 2,4-D mineral medium, we evaluated whether strain 712 could shift

to a 2,4-D pathway intermediate, succinate, in the same mineral medium. This occurred without a significant lag (Fig. 8) and produced a rapid doubling time (1.4 h). Growth in 2,4-D and transfer to 2,4-D medium resulted in continuous growth. Growth of an inoculum in Luria broth containing succinate resulted in significant lag periods after the culture was shifted to medium containing 2,4-D, suggesting that induction of the 2,4-D pathway may have been delayed. When culture filtrate from mid-log-phase cells of 2,4-D-grown strain 745, a member of the stimulatory triculture, was added to mineral medium containing 10% (vol/vol) 2,4-D, the lag time of *P. pickettii* 712 was reduced by one-third. While this procedure did not result in the shorter lag time found for the triculture, our findings do suggest that products of strain 745 do benefit this slowly growing and otherwise noncompetitive strain.

## DISCUSSION

We evaluated competition in three different environments (nonsterile soil, mixed broth cultures and axenic cultures) by

TABLE 2. Growth characteristics of 2,4-D-degrading bacteria in axenic broth cultures<sup>a</sup>

Strain	Lag time (h)	Specific growth rate ( $h^{-1}$ )	Relative fitness coefficient <sup>b</sup>
745	<15	0.173	1.0
<i>P. cepacia</i> DBO1(pJP4)	<15	0.179	0.95
K17	<15	0.185	0.74
<i>S. paucimobilis</i> 1443	25–40	0.181	0.03
<i>P. cepacia</i> DBO1(pKA4)	25–40	0.077	0.05
<i>P. pickettii</i> 712(pKA4)	>60	0.078	~0

<sup>a</sup> All values are means determined by using two independent broth cultures.

<sup>b</sup> The relative fitness coefficient for each strain was determined in an axenic broth culture by using the method of Lenski et al. (13); it was defined as the ratio of the number of doublings of a strain to the number of doublings of strain 745 under the same conditions. The first 30 h of incubation was chosen to evaluate relative fitness since the most rapidly growing strain, strain 745, stopped growing after 30 h because of depletion of substrate.

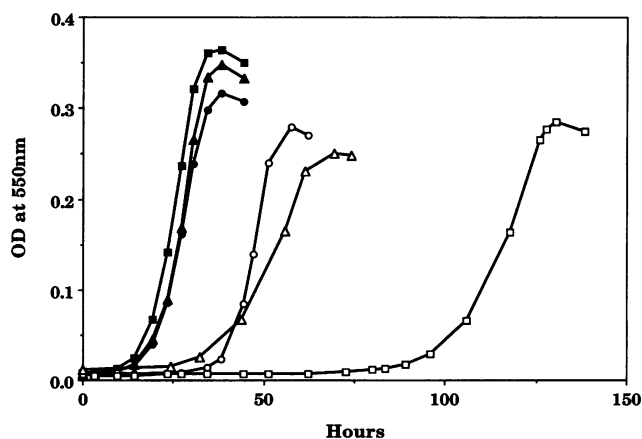


FIG. 6. Growth patterns of 2,4-D-degrading bacteria in axenic cultures. Symbols: ■, strain 745; ▲, *P. cepacia* DBO1(pJP4); ●, strain K17; ○, *S. paucimobilis* 1443; △, *P. cepacia* DBO1(pKA4); □, *P. pickettii* 712(pKA4). Each point is the mean for two replicate broth cultures. OD, optical density.

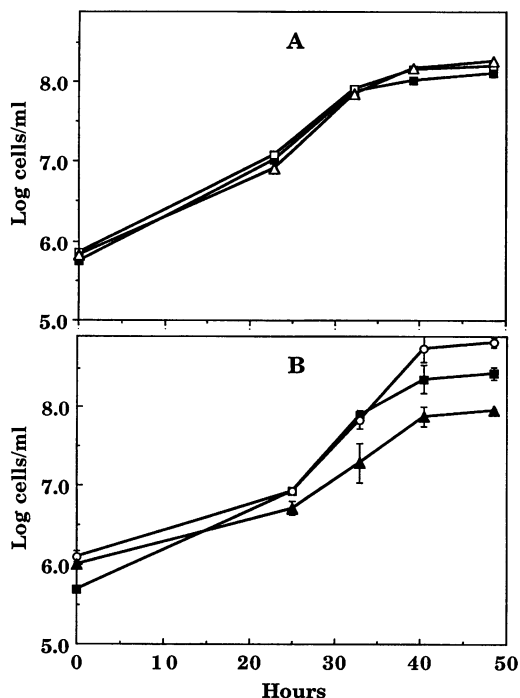


FIG. 7. (A) Competition for 2,4-D among strain 745, (■), *P. cepacia* DBO1(pJP4) (□), and K17 (△) in mixed liquid cultures. (B) Competition for 2,4-D among strain 745 (■), *S. paucimobilis* 1443 (○), and *P. pickettii* 712 (▲) in mixed liquid cultures. The data are the means and standard deviations for three replicate broth cultures.

using strains that originated from different habitats, including a laboratory host strain containing a plasmid isolated in Australia (1), three strains isolated from Michigan agricultural soils, and one strain indigenous to native Kansas prairie soil. The key features of these strains and their competitive perfor-

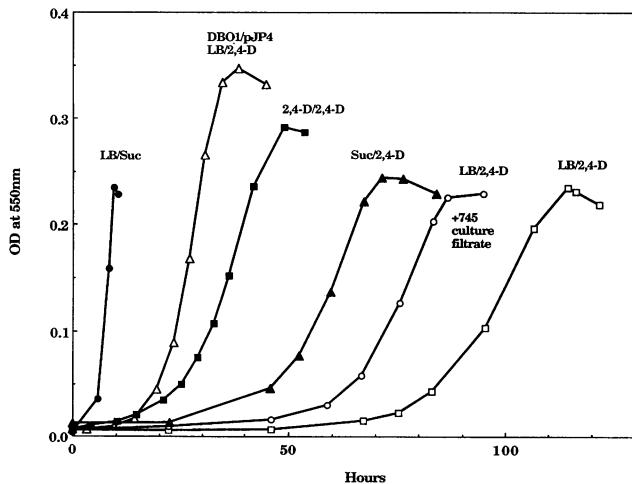


FIG. 8. Effect of culture conditions on the growth of *P. pickettii* 712. The lines are labelled with the medium on which the inoculum was grown, followed by the growth medium of the experiment. The results obtained for *P. cepacia* DBO1(pJP4) are shown for comparison. Culture filtrate from a strain 745 culture was added to the 2,4-D medium where indicated. Each point is the mean for replicate broth cultures. LB, Luria broth; Suc, succinate; OD, optical density.

mance characteristics in three different environments are summarized in Table 3. Kinetic parameters such as the maximum growth rate and  $K_s$ , which underlie the growth rate, are well-documented factors that are important to competition; this has recently been illustrated for 2,4-D-degrading strains (5). However, other factors, such as induction period, adaptation to the environment, and cross-feeding of growth factors, can also contribute to competitive outcome and were the foci of this study.

When four different 2,4-D-degrading strains were added at equal densities to a soil from a climatic region different than the region from which they originated, primary and secondary dominant strains emerged, an outcome not predicted from the fitness coefficients determined in axenic broth cultures. Strain 745, *P. cepacia* DBO1(pJP4), and indigenous strain K17 belong to the fast-growing group and thus were expected to outgrow both of the slowly growing bacteria, *P. pickettii* 712 and *S. paucimobilis* 1443, in soil. Although strain 745 had the highest fitness coefficient among these strains, it was not detected in soil competition experiments. Instead, the constructed strain, *P. cepacia* DBO1(pJP4), was observed as the dominant strain as determined by both the plate analysis and the hybridization results (Table 3). K17, the indigenous 2,4-D-degrading bacterium in Konza Prairie soil, was not detected in the inoculated soil throughout 10 2,4-D treatments, whereas this strain was dominant in the uninoculated soil after the fifth treatment. Considering that this strain has presumably never been exposed to 2,4-D in the past and that its initial population density was lower by a factor of 50, it apparently had no chance for detectable growth because *P. cepacia* DBO1(pJP4) and *S. paucimobilis* 1443 probably consumed most of the 2,4-D. This outcome was not necessarily expected since K17 did have the highest specific growth rate in medium containing 2,4-D and should have been well adapted to the other conditions of its native habitat.

*S. paucimobilis* 1443 was detected as a secondary dominant population in inoculated soil throughout this study (Table 3). This strain exhibited an intermediate lag time in broth cultures, which was followed by a specific growth rate that was similar to the specific growth rates of strain 745, *P. cepacia* DBO1(pJP4), and K17. This suggests that *S. paucimobilis* 1443 may grow slowly initially but has the potential to catch up with other fast-growing 2,4-D degraders. This potential was demonstrated in mixed broth cultures, in which *S. paucimobilis* 1443 outgrew strain 745 during the log phase, and may also have resulted in the secondary dominance of *S. paucimobilis* 1443 in the soil competition experiment. *P. pickettii* 712, which exhibited a long lag time followed by slow growth in axenic cultures, was not detected throughout this study in inoculated soil. It is also noteworthy that *S. paucimobilis* 1443 exhibits no DNA sequence homology with the *tfd* genes that were presumably important to the competitive growth of *P. cepacia* DBO1(pJP4), as well as to the lesser growth of the other strains. In a companion study, *S. paucimobilis* outcompeted *P. pickettii* 712 in their native Michigan soil, but these two strains were superior to all other strains (10), some of which harbor pJP4-like sequences (9). This result shows that *P. pickettii* 712 is competitive in some environments despite its poor performance in this study.

After the second 2,4-D treatment of uninoculated soil, the population density of indigenous 2,4-D-degrading bacteria reached levels which should have been detected by DNA probes. However, no significant hybridization to the *tfdA* probe was detected after the second treatment, and after the fifth treatment bands of weak intensity were observed in two of three microcosms and a strong band was seen in the third (Fig.



TABLE 3. Summary of key features of 2,4-D-degrading strains and the outcome of competition in different environments

Strain (plasmid)	Source	Hybridization to <i>tfd</i> genes	Competitive rank <sup>a</sup>		
			Axenic broth	Mixed broth	Soil
<i>P. cepacia</i> DBO1(pJP4)	Constructed	<i>tfdA, tfdB, tfdC, tfdD</i>	1	—	1
745(pBS5)	Michigan soil	<i>tfdA, tfdB, tfdC, tfdD</i>	1	2	0
K17(pKO51)	Native soil	<i>tfdA, tfdB, tfdC, tfdD</i>	1	—	0
<i>S. paucimobilis</i> 1443(pBS3)	Michigan soil	None	2	1	2
<i>P. pickettii</i> 712(pKA4)	Michigan soil	<i>tfdA</i>	3	3	0

<sup>a</sup> 0, present but not detected; —, not included in the triculture competition experiment.

5). This suggests that another 2,4-D-degrading microbial population not detected with the available gene probes was dominant in the initial phase and was replaced by (or became coestablished with) strain K17 after the fifth treatment with 2,4-D.

The importance of interactions among competing organisms for the outcome of competition was apparent when we compared the patterns obtained for the axenic broth cultures with the results obtained for the mixed broth cultures (Table 3). *S. paucimobilis* 1443 and *P. pickettii* 712 began to grow much earlier in mixed cultures with strain 745 than in axenic cultures, suggesting that some intermediates produced from strain 745 stimulated the growth of these two strains in mixed cultures. One possible explanation is that pathway intermediates induced the 2,4-D-degrading enzymes of the two less fit strains, thereby substantially reducing the long lag times. Another possible explanation is that nutritional factors provided by cross-feeding aided growth on this less favorable substrate. Lag periods before 2,4-D degradation in soil are typical; Loos (14) suggested that this could be due to enzyme induction, while Miwa and Kuwatsuka (16) felt that it was related to the small initial populations of 2,4-D degraders.

An interesting result of these studies is how important lag time can be in determining fitness and also how lag time can be compensated for by population interactions. The difference in the outcome of competition in the broth and soil experiments may have been due to the fact that the rapid exchange of cell products in the mixed broth environment reduced the fitness difference, while this did not occur in the unmixed, physically isolated niches present in the soil environment.

The impacts of different 2,4-D-degradative plasmids on the 2,4-D growth patterns of the host microorganisms were noticeable. For example, *P. cepacia* DBO1 carrying pJP4 exhibited a short lag period followed by rapid growth, but the same strain carrying pKA4 instead of pJP4 exhibited a relatively long lag period followed by slow growth (Fig. 6). The specific growth rate of *P. cepacia* DBO1(pKA4) ( $0.077 \text{ h}^{-1}$ ) was similar to that of *P. pickettii* 712(pKA4) ( $0.078 \text{ h}^{-1}$ ) but not to that of *P. cepacia* DBO1(pJP4) ( $0.179 \text{ h}^{-1}$ ). However, the lag time of *P. cepacia* DBO1(pKA4) was intermediate between the lag time of *P. cepacia* DBO1(pJP4) and the lag time of *P. pickettii*(pKA4). Thus, the plasmid and plasmid-host interactions determined the growth rate and lag time, respectively, which are key determinants of the competitive outcome.

Our results demonstrate that a xenobiotic compound can provide a useful model for studying competition in soil. Our most interesting findings were that species interactions appeared to affect competition to different degrees in soil and broth, perhaps making data derived from broth cultures less predictive for the soil habitat; that superior competitiveness was determined primarily by the plasmid; and that nonnative

soil strains and, in fact, a constructed strain that did not even originate from soil were the most successful competitors in soil.

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